TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	9/20/2016
Department:	Core Lab	**Due Date:**	10/12/2016
		Implementation:	**10/12/2016**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Kleihauer Betke SGAH.H13 v3

Description of change(s):

SOP:

Section	Reason
4,5,6	Remove individual section labeling instructions and add general one
4.2	Specify stored in flammable cabinet
10.6	Review data moved from section 6
10.7	Add reporting of percent fetal cells
15	Update to new standard wording
17	Update PI revision date

This revised SOP will be implemented on October 12, 2016

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Kleihauer Betke**	
Prepared by	Marjan Ahmadi, Ashkan Chini	Date: 8/17/2012
Owner	Robert SanLuis	Date: 8/17/2012

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

Form revised 2/02/2007

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Kleihauer Betke	Fetal Hemoglobin-Differential Staining Kit	KBT

Synonyms/Abbreviations
KBT, Fetal Cell Screen

Department
Hematology

Form revised 2/02/2007

2. ANALYTICAL PRINCIPLE

Fetal hemoglobin is both alkali and acid resistant. Consequently, it is not eluted from fresh blood slides fixed in an ethanol/phosphate buffer. After this treatment, cells containing fetal hemoglobin stain with eosin, while those cells without any Hb F appear as colorless ghosts.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Maternal blood should be collected as soon as possible following delivery or sensitizing event. Collect the sample in EDTA. Do not use cord blood.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria		
Type -Preferred	Whole Blood EDTA	
-Other Acceptable	None	
Collection Container	Lavender top tube	
Volume - Optimum	5.0 ml	
- Minimum	1.0 ml	
Transport Container and Temperature	Collection container at room temperature	
Stability & Storage Requirements	Room Temperature:	6 hours
	Refrigerated:	24 hours
	Frozen:	Unacceptable
Timing Considerations	Smears should be prepared within 24 hours of blood collection.	
Unacceptable Specimens & Actions to Take	Cord blood or serum samples, Clotted Samples, and specimens collected after RhIg administration. Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.	

Criteria	
Compromising Physical Characteristics	Gross hemolysis. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
Other Considerations	Prior to testing, mix the blood tube gently by inversion several times to ensure a homogenous sample.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
Ethanol Fixative (80% solution)	ENG Scientific Inc, Cat. No. 5990
Citric Acid Phosphate Buffer solution	ENG Scientific Inc, Cat. No. 9907
Mayer's Acid Alum Hematoxylin Stain	ENG Scientific Inc, Cat. No. 8550
Eosin Y Staining solution (0.1% aqueous)	ENG Scientific Inc, Cat. No. 8905

4.2 Reagent Preparation and Storage

Reagent	Ethanol Fixative (80% solution)
Container	Individual solution bottles
Storage	Room Temperature, kept in flammable cabinet
Stability	Both sealed and unsealed reagents are stable until expiration date stamped on the bottle.
Preparation	Reagent is ready for use. No preparation is required.

Reagent	Citric Acid Phosphate Buffer solution
Container	Individual solution bottles
Storage	Room Temperature
Stability	Both sealed and unsealed reagents are stable until expiration date stamped on the bottle.
Preparation	Reagent is ready for use. No preparation is required.

Reagent	Mayer's Acid Alum Hematoxylin Stain
Container	Individual solution bottles
Storage	Room Temperature
Stability	Both sealed and unsealed reagents are stable until expiration date stamped on the bottle.
Preparation	Reagent is ready for use. No preparation is required.

Reagent	Eosin Y Staining solution (0.1% aqueous)
Container	Individual solution bottles
Storage	Room Temperature
Stability	Both sealed and unsealed reagents are stable until expiration date stamped on the bottle.
Preparation	Reagent is ready for use. No preparation is required.

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Positive and Negative	Obtained from in house patients or specimens

6.2 Control Preparation and Storage

Control	Positive Control
Preparation	Obtain a fresh cord blood specimen located in Blood Bank. Place 2 drops of the cord blood and 2 drops of a male patient's blood (fresh EDTA sample preferred) in a 12 x 75mm glass tube. Mix and prepare two thin smears and let them air dry.
Storage/Stability	Slides are routinely prepared with each patient test performed, but are stable for 2 weeks when stored in the freezer at -20C or colder.

Control	Negative Control
Preparation	Use a male patient's fresh EDTA specimen and prepare two thin blood smears. Let them air dry.
Storage/Stability	Slides are routinely prepared with each patient test performed, but are stable for 2 weeks when stored in the freezer at -20C or colder.

6.3 Frequency

Both positive and negative controls are performed with each patient testing.

6.4 Tolerance Limits and Criteria for Acceptable QC

IF the QC result is …	THEN…
not reacting as expected (see section 10.1 for explanation)	repeat using new cord and male patient's blood; and fresh stain

- All corrective action must be documented as outlined in the Laboratory Quality Control Program.
- If repeating test does not produce acceptable QC, notify the supervisor immediately.
- No patient results are to be reported until acceptable QC results are obtained.

6.5 Documentation

- Quality Control is documented on the Kleihauer Betke Quality Control Log.
- Quality control records are reviewed daily at the bench, weekly by the Lead Technologist or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each KBT test or batch of KBT tests must be tested with positive and negative control materials.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Consult the Laboratory QC program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

- Freezer capable of sustaining range of -20°C or colder.
- Microscope
- Timer

7.3 Supplies

- 12 x 75 mm glass tubes
- Glass slides
- Pencil or solvent resistant marking pen
- Saline
- Staining rack

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Test Run
1.	**Patient Specimen**: Into a 12 x 75mm glass tube add 2 drops of maternal blood from a lavender top tube and 3 drops of saline. Mix and prepare 2 thin blood films. Label with patient name and accession number. Let slides air dry. **Controls**: Prepare Positive and Negative controls as described in Section 6.2. **Note**: Two slides are prepared for each patient or control. Only one slide is routinely read, the second slide is a backup in the event the first is poorly stained.
2.	Place the slides (controls and patient) on the slide staining rack above the sink in hematology.
3.	Flood the slides with Solution I (fixative). Allow to stand for 5 minutes.
4.	Rinse gently with tap water.
5.	Let slides air dry.
6.	Flood the slides with Solution II (citric acid/phosphate buffer). Allow to stand for 10 minutes.
7.	Rinse gently with tap water.
8.	Flood the slides with Solution III (Mayer's Acid Alum Hematoxylin Stain). Allow to stand 4 minutes.
9.	Rinse gently with tap water.
10	Flood the slides with Solution IV (Eosin Y, 0.1% aqueous solution). Allow to stand 15 seconds to 2 minutes.
11.	Rinse gently with tap water.
12.	Let slides air dry.
13.	Examine smears as specified in section 10.1

9. CALCULATIONS

$$\% \text{ fetal cells} = \frac{\text{Total fetal RBCs}}{\text{Total adult RBCs}} \times 100$$

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

The smears should be read as soon as possible after drying. This test is considered a STAT test and reporting a positive fetal bleed as soon as possible is paramount. A 100X microscope should be used for optimal cellular contrast and counting. Scan for the area of the slide with approximately 200-300 maternal cells touching but not stacked with the aid of a 40X ocular.

1. Scan the stained control slides at 400X magnification to determine the presence of fetal and non-fetal cells. The positive control should have both dark staining fetal and ghost non-fetal cells present. The negative should not have any of the fetal cells there; should only be ghost cells present. Record the results of the controls and patient(s) on the worksheet and place in the appropriate binder.
2. Scan the stained patient smear at 400X magnification to determine the area in which an even, but not scanty, distribution of cells is seen. **Count at 1000X magnification**.
3. Within this area, begin reading for a total of ten consecutive fields (*do not view the smear through the microscope to select the fields, for this may skew the count*).
4. Count the number of fetal erythrocytes and the number of adult erythrocytes in each field. Record the count of fetal erythrocytes and the number of adult erythrocytes in each field.
5. Total the number of fetal red blood cells observed in the ten fields.
6. Total the number of adult red blood cells observed in the same ten fields.
7. Calculate the ratio of fetal red blood cells to adult red blood cells by dividing the total number of fetal red blood cells observed in the ten microscope fields by the total number of adult red blood cells observed in the same ten microscope fields. Then multiply the ratio by 100 to get percentage.

Example:

Microscope field	Fetal Cells	Adult cells
1	2	245
2	1	247
3	0	242
4	1	252
5	2	253
6	1	245
7	2	262
8	1	262
9	1	200
10	0	220
Total cells counted	11	2408

Cell ratio = 11÷ 2408 = 0.0045
0.0045 x 100 = 0.45 %

10.2 Determining RhIG Dosage

Sunquest will automatically calculate the recommended number of vials of RhIG to give to Rh-negative or weak D positive patients. RhIG is not indicated for Rh-positive patients.

% Fetal Cells	RhIG Vials to Inject
<0.3	1
0.3 – 0.8	2
0.9 – 1.4	3
1.5 – 2.0	4
2.1 – 2.6	5
2.7 – 3.2	6
3.3 – 3.8	7
3.9 – 4.4	8
4.5 – 5.0	9
5.1 – 5.6	10
5.7 – 6.2	11
6.3 – 6.8	12
6.9 – 7.4	13
7.5 – 8.0	14
8.1 – 8.6	15

10.3 Rounding

N/A

10.4 Units of Measure

%

10.5 Clinically Reportable Range (CRR)

N/A

10.6 Review Patient Data

Check for unusual patterns, trends, or distributions in patient results (such as an unusually high percentage of positive results). Resolve any problems noted before issuing patient reports.

10.7 Repeat Criteria and Resulting

Reporting Results:
All reports are entered into the computer system via manual entry.
Function: MEM
Worksheet: SHE1

IF the result is …	THEN…
No fetal cells are detected	Result using English Text Code - **NFET**
Fetal cells seen	Result the percent to 2 decimals places as calculated in section 10.1

Notify the patient care area if testing results indicate the patient is Rh-negative or weak D positive **AND** requires more than 1 vial of RhIG.

11. EXPECTED VALUES

11.1 Reference Ranges

No fetal cells detected

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

The cytological detection of cells containing fetal hemoglobin is of importance in determining the distribution of fetal hemoglobin in red cells. It is also useful in determining the presence of fetal red blood cells in the maternal circulation, which assesses the magnitude of fetal maternal hemorrhage and enables calculation of the dosage of Rh immune globulin to be given.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

- Perform test immediately after delivery to minimize effect of ABO incompatibility.
- Timing is critical in fixing, staining and eluting the smears.
- Oil immersion lens must not be used to *scan* for the proper counting area.

- When counting, do not use a bright microscope light to increase the contrast between adult and fetal cells.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

N/A

14.3 Interfering Substances

Maternal antibody reactive with fetal A and B antigens may remove many of the fetal cells

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. Laboratory Safety Manual
3. Safety Data Sheets (SDS)
4. Quest Diagnostics Records Management Procedure
5. Hemolysis, Icteria and Lipemia Interference (Lab policy)
6. Repeat Testing Requirement (Lab policy)
7. Kleihauer Betke Quality Control Log (AG.F116)
8. Current package insert of ENG Scientific, Inc

17. REFERENCES

1. ENG Scientific, Inc, Fetal Hemoglobin-Differential Staining Kit, Package Insert, revised 01/2014
2. Sure-Tech Diagnostic Assoc., Inc., Kleihauer-Betke Fetal Hemoglobin Reference Manual, revised 1996

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SGAH.H 014.001		
000	3.18.2013	10.2	Added RhIG dosing information	SCodina	NCacciabeve
001	7.25.2014	10.2	Added comment indicating RhIG is not indicated in Rh-positive patients.	S Codina	R SanLuis
001	7.25.2014	10.6	Added requirement to call results when more than 1 vial of RhIG is indicated in an Rh-neg/weak D pos patient.	S Codina	R SanLuis
001	7.25.2014	16	Form moved from section 19	L Barrett	R SanLuis
001	7.25.2014	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis
2	8.26.2016	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
2	8.26.2016	4.2	Specify stored in flammable cabinet	L Barrett	R SanLuis
2	8.26.2016	10.6	Review data moved from section 6	L Barrett	R SanLuis
2	8.26.2016	10.7	Add reporting of percent fetal cells	L Barrett	R SanLuis
2	8.26.2016	15	Update to new standard wording	L Barrett	R SanLuis
2	8.26.2016	17	Update PI revision date	L Barrett	R SanLuis

19. ADDENDA

None